# DATA EVALUATION RECORD

FLUTRIAFOL (PP450)

Study Type: OPPTS 870.3700b [§83-3b]; Developmental Toxicity Study in Rabbits

Work Assignment No. 5-01-151 E; formerly 4-01-151 E (MRID 47090350)

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## DATA EVALUATION RECORD

**STUDY TYPE:** Prenatal Developmental Toxicity Study – Rabbit (gavage); OPPTS 870.3700b [§83-3a]; OECD 414.

**PC CODE:** 128940 **DP BARCODE:** D340368
**TXR#:** 0054780 **DECISION:** 377412

**TEST MATERIAL (PURITY):** Flutriafol Technical (93% a.i.)

**SYNONYMS:** PP450; α-(2-fluorophenyl)-α-(4-fluorophenyl)-1*H*-1,2,4-triazole-1-ethanol

**CITATION:** PP450 (Flutriafol): Teratogencity study in the rabbit. Imperial Chemical Industries PLC, Cheshire, UK. Laboratory Study No. RR0214; Report No. CTL/P/747, October 20, 1982. MRID 47090350. Unpublished.

**SUBMITTER/SPONSOR:** Cheminova Inc., 1600 Wilson Boulevard, Suite 700, Arlington, VA (originally sponsored by Imperial Chemical Industries PLC)

**EXECUTIVE SUMMARY:** In a developmental toxicity study (MRID 47090350), Flutriafol (PP450; 93%; Batch # P10) was administered daily in gelatin capsules to 18 presumed pregnant Dutch rabbits at doses of 0, 2.5, 7.5, or 15 mg/kg/day from gestation days (GD) 6-18. On GD 29, each surviving female was euthanized, and the uterus was removed via cesarean section and its contents examined. Fetuses were examined for external, visceral, and skeletal malformations and variations.

At 7.5 mg/kg/day, one doe (#52) aborted part of its litter on GD 20 and was removed from the study. At 15 mg/kg/day, one doe (#70) was killed *in extremis* after observations that the animal had not been eating or drinking and that it had lost weight and was in poor condition. No other maternal deaths could be attributed to treatment. Loose feces on the cage floor and/or fur of the animals was observed in 2/15 rabbits at 7.5 mg/kg/day and 4/15 rabbits at 15 mg/kg/day. These findings were observed only once per female, except for one doe at 7.5 mg/kg/day for which the observation was made on 2 days and one doe at 15 mg/kg/day for which loose feces was noted on three days. The observations were considered treatment-related but nonadverse.

At 15 mg/kg/day, maternal body weight gains were decreased during the treatment interval (-79 g

94

treated vs 48 g controls) and for the overall (GD 0-29) study, both when uncorrected for (149 g treated vs 230 g controls) and when corrected for (-158 g treated vs -55 g controls) gravid uterine weights. Additionally at this dose, maternal food consumption was increased by 24% ($p \leq 0.01$) over controls during the pre-treatment interval, but was decreased by 22% (not significant) during treatment. In two of the females examined at 15 mg/kg/day, the stomach was found to contain a fur ball and was otherwise empty or contained little food. Only a single female at 7.5 mg/kg/day had little to no food in the stomach. Additionally at 15 mg/kg/day, one of the aforementioned does had dark pitted areas on the mucosal surface of the glandular portion of the stomach.

**The maternal LOAEL is 15 mg/kg/day based on decreased corrected and uncorrected body weight gains and food consumption. The maternal NOAEL is 7.5 mg/kg/day.**

The number of early intrauterine deaths was higher at 15 mg/kg/day than controls (36 deaths; 31.0%) compared to controls (11 deaths; 10.4%). Similarly, the number of late intrauterine deaths was increased at this dose (19 deaths; 16.4%) compared to controls (1 death; 1.0%), as was the proportion of does affected with late intrauterine deaths (3/14 vs. 1/15 in controls). Complete litter resorptions were significantly higher ($p<=0.05$) at 15 mg/kg/day, occurring in 5/14 does compared to 0/15 controls. These findings resulted in a significantly increased ($p<=0.01$) post-implantation loss at 15 mg/kg/day (45.5% vs 13.1% controls); a decreased number of litters (9 vs 15); and a decreased total (61 vs 94) and mean (4.0 vs 6.5; $p<=0.05$) number of live fetuses.

There were no treatment-related effects on growth or development of the fetuses. Fetal body weights and litter weights of the treated groups were comparable to controls. Reduced/delayed ossification was observed in several bones in the skeleton (skull, vertebrae, and sternebrae) at an increased incidence over controls. However, these findings were minor in incidence and were not statistically significantly different from the controls. Furthermore, mean scores for ossification of the *manus* and *pes* in all treated groups were comparable to controls.

There were no treatment-related external, visceral, or skeletal malformations or variations. Two fetuses, one at 7.5 mg/kg/day and another at 15 mg/kg/day, had multiple abnormalities; however, historical control data showed that similar findings were previously noted in individual fetuses (e.g., cleft palate, gastroschisis, malformed eyes, and shortened/flexed limbs with reduced number of digits). Furthermore, the findings in the fetus at 7.5 mg/kg/day were more severe than those in the 15 mg/kg/day fetus. All other findings were unrelated to dose, minor in incidence, and/or not significantly different from the controls.

**The developmental LOAEL is 15 mg/kg/day based on decreased number of live fetuses, complete litter resorptions, and increased post-implantation loss. The developmental NOAEL is 7.5 mg/kg/day.**

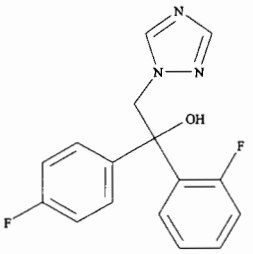
This study is classified **acceptable/guideline** and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in rabbits.

**COMPLIANCE:** Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided.

# I. MATERIALS AND METHODS

## A. MATERIALS

**1. Test material:** Flutriafol (PP450) Technical

| | |
|---|---|
| **Description:** | White solid |
| **Batch #:** | P10 |
| **Purity:** | 93% |
| **Compound stability:** | Not applicable because compound administered neat in capsules. |
| **CAS #:** | 76674-21-0 |
| **Structure:** |  |

**2. Vehicle:** Gelatin capsule

**3. Test animals**

| | | |
|---|---|---|
| **Species:** | Rabbit | |
| **Strain:** | Dutch | |
| **Age/weight at study initiation:** | Age not stated; 1.67-2.94 kg | |
| **Source:** | Ranch Rabbits (Crawley Down, Sussex, UK) | |
| **Housing:** | Individually in suspended metal cages | |
| **Diet:** | CRB Rabbit Pellets (Labsure Animal Diet, Poole, Dorset, UK), *ad libitum* | |
| **Water:** | Tap water, *ad libitum* | |
| **Environmental conditions:** | **Temperature:** | 15-22°C |
| | **Humidity:** | Not reported |
| | **Air changes:** | Not reported |
| | **Photoperiod:** | 12 hours light/12 hours dark |
| **Acclimation period:** | At least one week | |

## B. PROCEDURES AND STUDY DESIGN

**1. In life dates:** Start: February 15, 1982 End: May 20, 1982

**2. Mating:** Eight male Dutch rabbits were supplied by the same breeder. Each adult nulliparous female rabbit was paired with a male, and coitus was observed twice. The fertility of each buck was confirmed by taking a vaginal smear from the first doe with which it was mated and examining the smear for the presence of motile sperm. Within 2.5 hours after the second mating, each doe was given an intravenous injection of chorionic gonadotrophin to ensure ovulation. The day of mating was designated as gestation day (GD) 0. Twelve females (3 per group) were mated daily, on six days over a two week period. On the last day of mating, it was necessary to mate 13 females instead of 12. This was due to the removal of one female from the study on GD 8 due to ill-health, unrelated to treatment.

3. **Animal assignment:** Initially, the mated females were randomly assigned to the dose groups shown in Table 1. The cages were then arranged randomly but in replicates on the racks, so that each replicate contained one animal from each group. The extra female mated for the 15 mg/kg/day group was housed on an additional rack Later allocations were not strictly random, but instead distributed any females mated with the same male evenly among the groups.

| TABLE 1. Animal Assignment [a] | | | | |
|---|---|---|---|---|
| Dose (mg/kg/day) | 0 | 2.5 | 7.5 | 15 |
| No. females | 18 | 18 | 18 | 19 [b] |

a Data were obtained from pages 12-13 of the study report.

b An additional female (#73) was mated for this group due to the removal of one doe from this group on GD 8 due to ill health unrelated to treatment.

4. **Dose selection rationale:** The dose levels were selected based on the results of preliminary work which included an embryotoxicity study at dose levels of 5, 10, and 15 mg/kg/day. At 15 mg/kg/day, post-implantation loss was increased, and fetal body weights were decreased. No further information was provided.

5. **Dose preparation, administration, and analysis:** The test material was administered in gelatin capsules daily from gestation days (GD) 6-18. The actual weight of the compound contained in each capsule was dependent on the dose level and the body weight range for which the capsule was being prepared. Body weight ranges were defined to cover the weights of all rabbits in the study, and the capsules were prepared for the mid point of each range. The weight difference in each of the 9 body weight ranges was 0.2 kg (i.e., 1.6-1.8, 1.8-2.0,..., and 3.2-3.4 kg). Capsule content limits were also defined for each weight range and dose level. It was stated that the maximum error using the defined weight ranges and capsule content limits was calculated to be ±11.6% (88.4-111.6% nominal). This was for the lightest rabbit from the 2.5 mg/kg/day group being dosed with the heaviest capsule, from the lowest weight range. The accuracy of the methods of preparation was improved with heavier rabbits and increasing dose level. Frequency of preparation and storage conditions were not reported.

**Results** – It was stated that the dose received by each female was within ±10% nominal, with the exception of one 2.5 mg/kg/day female and one 15 mg/kg/day female which did not swallow the capsule on one day of dosing. The reviewers consider the difference between the nominal and actual dosage to the animals to be acceptable.

## C. OBSERVATIONS

1. **Maternal observations and evaluations:** All animals were checked daily throughout the study for mortality and clinical signs of toxicity. Body weights were recorded on GD 0, 6-19 (inclusive), and 29. Body weight gains were reported for GD 0-6 (pre-treatment), 6-19 (treatment), and 19-29 (post-treatment) intervals. Additionally, body weight gains for the overall study (GD 0-29) were calculated by the reviewers by adding the body weight gains for each of the above listed intervals in the study; and net overall body weight gains (corrected for gravid uterine weight) were calculated by the reviewers by subtracting the weight of the gravid uterus from the body weight gain for GD 0-21. Individual food consumption (g/rabbit) was determined for the pre-treatment (GD 0-6), treatment (6-19), and post-treatment (19-29) intervals. On GD 29, the females were euthanized by an intravenous injection of sodium pentobarbitone solution in the marginal ear vein. Gross necropsies were performed on all animals, including those found dead or removed from study prior to study termination. Prior to examination of the maternal organs, a cesarean section was performed, and the gravid uterus was removed and weighed. The number of corpora lutea in each ovary was counted. The uterine contents were then examined, and the numbers of live fetuses, early intrauterine deaths, and late intrauterine deaths were counted. Late intrauterine deaths were characterized by distinguishable fetal tissue.

2. **Fetal evaluations:** After recording the position of each live fetus in the uterus, the fetuses were removed in sequence (starting at the ovarian end of the left horn and ending at the ovarian end of the right horn), weighed, and euthanized by an intracardiac injection of sodium pentobarbitone solution. All fetuses were examined for external malformations and then dissected, examined for visceral abnormalities, and skinned. The fetuses were then eviscerated and the carcasses fixed in 70% methanol. The following day, the heads were cut through the fronto-parietal suture, and the brain was examined for macroscopic abnormalities. Following examination, the carcasses were returned to 70% methanol prior to processing and staining with Alizarin Red S using the method of Staples and Schnell (1964). The stained fetal skeletons were examined for abnormalities, and the degree of ossification was assessed. The individual bones of the hand (*manus*) and foot (*pes*) were assessed, and the results converted to a semi-quantitative five-point scale found in Appendix 6 on page 43 of the study report, included as an Attachment to this DER. External, visceral, and skeletal abnormalities were classified as major (rare and/or lethal) or minor (deviations from normal that are common).

## D. DATA ANALYSIS

1. **Statistical analyses:** The analysis of variance (ANOVA) allowed for the replicate design of the study. Individual group means were adjusted for missing values prior to pair-wise comparison of the treated groups with the controls using Student's t-test. All statistical tests were one-sided, with the following exceptions which were two-sided: body weight gain; food consumption, the number of corpora lutea; and the proportion of male fetuses. Significance was denoted at $p \leq 0.05$ and $p \leq 0.01$. It was not stated whether the assumptions of homogenous variances and normal distribution of the data were tested prior to proceeding

with parametric analyses. Otherwise, the statistical analyses were considered appropriate.

| Parameter | Statistical test |
|---|---|
| Initial (GD 0) maternal body weight<br>Maternal body weight gains for GD 0-6, 6-19 and 19-29<br>Maternal food consumption for GD 0-6, 6-19 and 19-29<br>Numbers of corpora lutea, implantations, and live fetuses per doe<br>Gravid uterine weight, total litter weight, and mean fetal weight (calculated on an individual litter basis)<br>Mean *maus* and *pes* scores per fetus | Analysis of variance (ANOVA) followed by pair-wise comparison of the treated groups with the controls using Student's t-test |
| Percent pre-implantation loss and post-implantation loss<br>Percent fetuses with major and minor external and visceral defects, or major and minor skeletal defects and variants (calculated on an individual litter basis) | Double arc-sine transformation of Freeman and Tukey (1950) prior to ANOVA. Student's t-test was conducted for pair-wise comparison of the treated groups with the controls. |
| Proportions of females with pre-implantation loss, post-implantation loss, early intra-uterine deaths, late intrauterine deaths, and complete litter resorption<br>Proportion of male fetuses<br>Proportion of fetuses with major or minor external or visceral defects, and major and minor skeletal defects and variants (also analyzed on a litter basis) | Fisher's Exact Test for pair-wise comparison of treated groups with the controls |

2. **Indices:** The following indices were reported:

   Pre-implantation loss (%) = (# corpora lutea – # implantations)/ # corpora lutea x 100%

   Post-implantation loss (%) = (# implantations – # live fetuses)/ # implantations x 100%

   For pre-implantation loss, the difference between the number of corpora lutea and the number of implantations was assumed to be zero for females for which the corpora lutea count was exceeded by the number of implantations.

3. **Historical control data:** Historical control data from developmental toxicity studies conducted at the performing laboratory using the same strain of rabbit were provided. These data comprised incidences of selected external, visceral, and skeletal malformations and variations from 10 studies conducted from 1972-1982.

## II. RESULTS

## A. MATERNAL TOXICITY

### 1. Mortality and clinical observations

a. **Mortality:** Survival was unaffected by treatment with flutriafol. The number of females

killed *in extremis* or found dead was 1, 0, 2, and 2 in the control, 2.5, 7.5, and 15 mg/kg/day groups, respectively (Table 2). At 15 mg/kg/day, one doe (#70) was killed *in extremis* after observations that the animal had not been eating or drinking and that it had lost weight and was in poor condition. Additionally at this dose, another doe (#55) was removed from the study on GD 8 due to a large scab under its chin extending to mid thorax. This finding was considered unrelated to treatment. At 7.5 mg/kg/day, one doe (#52) aborted part of its litter on GD 20 and was removed from the study. Another doe at this dose, in addition to one control female, were found dead with no definitive cause of death. All other females survived until scheduled termination.

b. **Clinical signs of toxicity:** At 15 mg/kg/day, one doe (#61) was described as extremely subdued, very weak, shaky, lying on side in cage, and very thin on GD 29 (Table 2). Loose feces on the cage floor and/or fur of the animals was observed in 2/15 rabbits at 7.5 mg/kg/day and 4/15 rabbits at 15 mg/kg/day. These findings were observed only once per female, except for one doe at 7.5 mg/kg/day for which the observation was made on 2 days and one doe at 15 mg/kg/day for which loose feces was noted on three days. The observation of loose feces was considered treatment-related but non-adverse.

**TABLE 2. Maternal survival and selected clinical signs of toxicity (# affected)** [a]

| Clinical observation | Dose in mg/kg/day | | | |
|---|---|---|---|---|
| | 0 | 2.5 | 7.5 | 15 |
| Total found dead or killed prior to termination | 1 | 0 | 2 | 2 |
| Found dead | 1 [b] | 0 | 1 [b] | 0 |
| Aborted | 0 | 0 | 1 [c] | 0 |
| Removed from study (unrelated to treatment) | 0 | 0 | 0 | 1 [d] |
| Killed *in extremis* | 0 | 0 | 0 | 1 [e] |
| Thin, shaky/subdued, weak, lying on side | 0 | 0 | 0 | 1 [f] |
| Loose feces on cage floor and/or fur [g] | 0 | 0 | 2 | 4 |

a Data were obtained from the text on pages 17-18, Table 2 on page 23, and Appendix A on pages 46-59 of the study report; n = 15.
b Found dead with no definitive cause of death.
c Doe #52 was removed from the study on GD 20 because it aborted part of its litter.
d Doe #55 was removed from the study on GD 9 due to a large scab under its chin extending to mid thorax. It was considered that the wound may reopen with constant handling and cause the animal distress.
e Doe #70 was killed on GD 23 after observations of being very thin and shaky and not eating or drinking.
f Doe #61 was described as extremely subdued, very weak, shaky, lying on side in cage, and very thin on GD 29.
g These findings were observed only once per female, except for one doe at 7.5 mg/kg/day for which the observation was made on 2 days and one doe at 15 mg/kg/day for which loose feces was noted on three days.

2. **Body weight:** At 15 mg/kg/day, maternal body weight gains were decreased during the treatment interval (-79 g treated vs 48 g controls) and for the overall (GD 0-29) study, both when uncorrected for (149 g treated vs 230 g controls) and when corrected for (-158 g treated vs -55 g controls) gravid uterine weights (Table 3). Body weight gains for the pre-treatment and post-treatment intervals, in addition to gravid uterine weights, in this group were comparable to controls. Body weight gains in the 2.5 and 7.5 mg/kg/day females were comparable to controls throughout the study.

| TABLE 3. Mean maternal initial body weight and body weight gains (g) [a] | | | | | |
|---|---|---|---|---|---|
| Interval | | Dose in mg/kg/day [# does] [b] | | | |
| | | 0 [15] | 2.5 [16] | 7.5 [15] | 15 [14] |
| Initial body weight | GD 0 | 2109 | 2143 | 2172 | 2145 |
| Pre-treatment | GD 0-6 | 24 | 64 | 48 | 76 |
| Treatment | GD 6-19 | 48 | 33 | 34 | -79 |
| Post-treatment | GD 19-29 | 158 | 187 | 162 | 152 |
| Uncorrected weight gain (GD 0-29) [c] | | 230 | 284 | 244 | 149 |
| Gravid uterine weight [d] | | 285 | 316 | 286 | 307 |
| Corrected weight gain | (GD 0-29) [e] | -55 | -32 | -42 | -158 |

a Data were obtained from Table 4 on page 26 and Table 6 on page 29 of the study report.
b n = number of pregnant does.
c Calculated by the reviewers by adding the body weight gains for the pre-treatment, treatment, and post-treatment intervals.
d The mean gravid uterine weights were based on an n = 15, 15, 15, and 10 in the control, 2.5, 7.5, and 15 mg/kg/day groups, respectively. At 15 mg/kg/day, there were 10 uterus weights, although there were only 9 live litters because 1 uterus was weighed from a female whose litter died just prior to term. This litter was classified as a total resorption.
e Calculated by the reviewers by subtracting the mean gravid uterine weight from the mean overall (GD 0-29) gain.

3. **Food consumption:** At 15 mg/kg/day, maternal food consumption was increased by 24% ($p \leq 0.01$) over controls during the pre-treatment interval, but was decreased by 22% (not significant) during treatment (Table 4). Food consumption in this group was comparable to controls during post-treatment. Food consumption in the 2.5 and 7.5 mg/kg/day does was comparable to controls throughout the study.

| TABLE 4. Mean maternal food consumption (g/animal) [a] | | | | | |
|---|---|---|---|---|---|
| Interval | | Dose in mg/kg/day [# does] [b] | | | |
| | | 0 [14] | 2.5 [15] | 7.5 [15] | 15 [14] |
| Pre-treatment | GD 0-6 | 653 | 731 | 663 | 808** (↑24) |
| Treatment | GD 6-19 | 1192 | 1280 | 1156 | 935 (↓22) |
| Post-treatment | GD 19-29 | 1079 | 980 | 1062 | 1107 |

a Data were obtained from Table 5 on page 27 of the study report. Percent difference from controls, calculated by the reviewers, is included in parentheses.
b Number of does with live fetuses.
** Significantly different from the control group at $p \leq 0.01$

4. **Gross pathology:** In two of the females examined at 15 mg/kg/day (#61 and 70), the stomach was found to contain a fur ball and was otherwise empty or contained little food (Table 5). Only a single female at 7.5 mg/kg/day (#42) had little to no food in the stomach. Additionally at 15 mg/kg/day, one of the aforementioned does (#61) had dark pitted areas on the mucosal surface of the glandular portion of the stomach.

| TABLE 5. Incidence of selected macroscopic findings in does at necropsy (# affected) [a] | | | | |
|---|---|---|---|---|
| Gross finding | Dose in mg/kg/day | | | |
| | 0 | 2.5 | 7.5 | 15 |
| **Stomach** | | | | |
| **Empty/contained little food** | 0 | 0 | 1 | 2 [b,] |
| **Fur ball** | 0 | 0 | 0 | 2 [b,] |
| **Lesions on or in** | 0 | 0 | 0 | 1 [c] |

a Data were obtained from Table 3 on page 25 of the study report; n = 15.
b Stomach containing fur ball and otherwise empty/containing little food in Doe #61 and 70.
c Dark pitted areas on the mucosal surface of the glandular stomach was noted in Doe #61

5. **Cesarean section data:** Summary data from the cesarean sections are presented in Table 6. The number of early intrauterine deaths was higher at 15 mg/kg/day than controls (36 deaths; 31.0%) compared to controls (11 deaths; 10.4%). Similarly, the number of late intrauterine deaths was increased at this dose (19 deaths; 16.4%) compared to controls (1 death; 1.0%), as was the proportion of does affected with late intrauterine deaths (3/14 vs. 1/15 in controls). Complete litter resorptions were significantly higher ($p \leq 0.05$) at 15 mg/kg/day, occurring in 5/14 does compared to 0/15 controls. These findings resulted in a significantly increased ($p \leq 0.01$) post-implantation loss at 15 mg/kg/day (45.5% vs 13.1% controls); a decreased number of litters (9 vs 15); and a decreased total (61 vs 94) and mean (4.0 vs 6.5; $p \leq 0.05$) number of live fetuses. No other treatment-related findings were observed during the cesarean section examinations.

**TABLE 6. Cesarean section observations** [a]

| Observation | Dose (mg/kg/day) | | | |
|---|---|---|---|---|
| | 0 | 2.5 | 7.5 | 15 |
| No. Animals assigned (mated) | 18 | 18 | 18 | 19 |
| No. Pregnant | 15 | 16 | 15 | 14 |
| Pregnancy rate (%) [b] | 83.3 | 88.9 | 83.3 | 73.7 |
| No. Not pregnant | 2 | 2 | 3 | 5 |
| Maternal wastage | 1 | 0 | 2 | 2 |
| No. Died | 1 | 0 | 1 | 1 [c] |
| No. Died pregnant | 1 | 0 | 1 | 1 [c] |
| No. Died nonpregnant | 0 | 0 | 0 | 1 [d] |
| No. Aborted | 0 | 0 | 1 | 0 |
| No. Premature deliveries | 0 | 0 | 0 | 0 |
| Mean No. Corpora lutea | 9.0 | 8.5 | 7.9 | 9.9 |
| Mean No. Implantations | 7.1 | 7.0 | 6.4 | 8.1 |
| Total No. litters | 15 | 15 | 15 | 9 |
| Total No. live fetuses | 94 | 102 | 94 | 61 |
| Mean No. Live fetuses | 6.5 | 6.3 | 6.0 | 4.0* |
| Early intra-uterine deaths (No.) | 11 | 8 | 5 | 36 |
| Mean percent (%) | 10.4 | 7.1 | 5.0 | 31.0 |
| Proportion of does affected | 5/15 | 6/16 | 4/15 | 6/14 |
| Late intra-uterine deaths | 1 | 3 | 1 | 19 |
| Mean percent (%) | 1.0 | 2.7 | 1.0 | 16.4 |
| Proportion of does affected | 1/15 | 2/16 | 1/15 | 3/14 |
| Complete litter resorptions (proportion of does affected) | 0/15 | 1/16 | 0/15 | 5/14* |
| Mean gravid uterus weight (g) | 285 | 316 | 286 | 307 |
| Mean total litter weight | 208 | 224 | 208 | 223 |
| Mean live fetal weight (g) | 34.0 | 34.8 | 35.6 | 32.4 |
| Sex ratio (% male) | 55 | 52 | 48 | 46 |
| Pre-implantation loss (%) [e] | 20.9 | 13.4 | 17.5 | 8.7 |
| Proportion of does affected | 11/15 | 12/15 | 8/15 | 5/11 |
| Post-implantation loss (%) [f] | 13.1 | 13.8 | 5.8 | 45.5** [g] |
| Proportion of does affected | 6/15 | 8/16 | 4/15 | 8/14 |

a Data were obtained from text on pages 17-18, Table 6 on pages 28-29, Table 7 on page 30, Appendix A on pages 46-59, and Appendix C on pages 65-69 in the study report. Percent difference from the control group, calculated by the reviewers, is included in parentheses.

b Calculated by the reviewers from data presented in this table.

c Doe #70 was killed on GD 23 after observations of being very thin and shaky and not eating or drinking.

d Doe #55 was removed from the study on GD 9 due to a large scab under its chin extending to mid thorax. It was considered that the wound may reopen with constant handling and cause the animal distress.

e Pre-implantation loss (%) = (mean # corpora lutea – mean # implantations)/mean # corpora lutea x 100

f Post-implantation loss (%) = (mean # implantations – mean # live fetuses)/mean # implantations x 100

g Although statistics were performed on the double arc-sine transformed values, the reviewers denoted significance next to the untransformed data presented in this table.

* Significantly different from the controls at $p \leq 0.05$

** Significantly different from the controls at $p \leq 0.01$

## B. DEVELOPMENTAL TOXICITY

1. **External examination:** All external findings are presented in Table 7. There were no treatment-related external malformations (referred to as major defects in the study report) or variations (referred to as minor defects). Two fetuses, one at 7.5 mg/kg/day and another at 15 mg/kg/day, had multiple abnormalities. The following findings were observed in a single fetus at 7.5 mg/kg/day (Fetus B, Doe #45): (i) upper jaws shortened; (ii) cleft palate; (iii) right eye open; (iv) left eye microphthalmia; (v) polex absent (bilateral); (vi) clubbed right hindfoot; and (vii) abnormally shaped left hindlimb with a reduced number of digits. The following findings were observed in a single fetus (Fetus A, Doe #57) at 15 mg/kg/day: (i) shortened upper jaw; (ii) bilateral clubbed hindfeet; (iii) shortened left forearm; and (iii) flexion of forefoot. Vestigial tail, a variation, was observed in four fetuses in a single litter at 2.5 mg/kg/day. Other variations of flexion of forefeet and hindfeet and malrotation of hindlimbs were noted in individual fetuses per finding at 2.5 mg/kg/day. There were no other external findings.

**TABLE 7. All external findings (# fetuses affected)** [a]

| Observation | Dose (mg/kg/day) | | | |
|---|---|---|---|---|
| | 0 | 2.5 | 7.5 | 15 |
| No. Fetuses (litters) examined | 94 (15) | 102 (15) | 94 (15) | 61 (9) |
| Malformations (major defects) | | | | |
| Upper jaw shortened | --- | --- | 1 [b] | 1 [c] |
| Cleft palate | --- | --- | 1 [b] | --- |
| Right eye open | --- | --- | 1 [b] | --- |
| Left eye microphthalmia | --- | --- | 1 [b] | --- |
| Polex absent - bilateral | --- | --- | 1 [b] | --- |
| Clubbed hindfoot - right | --- | --- | 1 [b] | --- |
| Clubbed hindfeet - bilateral | --- | --- | --- | 1 [c] |
| Left forearm shortened | --- | --- | --- | 1 [c] |
| Left hindlimb, reduced number of digits and abnormally shaped | --- | --- | 1 [b] | --- |
| Variations (minor defects) | | | | |
| Vestigial tail | --- | 4 [d] | --- | --- |
| Flexion of forefoot | --- | --- | 1 [b] | 1 [c] |
| Flexion of forefeet | --- | 1 | --- | --- |
| Flexion of hindfeet | --- | 1 | --- | --- |
| Malrotated hindlimbs | --- | 1 | --- | --- |

a Data were obtained from Tables 7 and 8 on pages 30-32 and from individual data on pages 97-99, 104-105, and 112 of the study report.
b All findings denoted by this subscript were observed in the same fetus (Fetus B, Doe #45)..
c All findings denoted by this subscript were observed in the same fetus (Fetus A, Doe #57).
d All fetuses with vestigial tail were observed in the same litter (Doe #36).
--- No animals affected (i.e., zero incidence)

2. **Visceral examination:** All visceral findings are presented in Table 8. There were no treatment-related visceral malformations or variations. Encephalocoele and gastroschisis were observed in one 7.5 mg/kg/day fetus and one 15 mg/kg/day fetus. Several variations were observed, including hemorrhage of the blood vessels surrounding the urinary bladder and dilatation of the lateral and mid brain ventricles, kidney pelvis(es), and ureters. However, these findings were minor in incidence and severity and/or were not dose-related.

**TABLE 8. All visceral findings (# fetuses affected) [a]**

| Observation | Dose (mg/kg/day) | | | |
|---|---|---|---|---|
| | 0 | 2.5 | 7.5 | 15 |
| No. Fetuses (litters) examined | 94 (15) | 102 (15) | 94 (15) | 61 (9) |
| Malformations (major defects) | | | | |
| Encephalocoele | --- | --- | 1 [b] | 1 [c] |
| Gastroschisis | --- | --- | 1 [b] | 1 [c] |
| Variations (minor defects) | | | | |
| Hemorrhage of blood vessels surrounding urinary bladder | 1 | 1 | 1 | 2 |
| Lateral brain ventricles dilated (slight) | 2 | --- | --- | 1 |
| (moderate) | --- | --- | --- | 1 |
| Mid brain ventricle dilated (moderate) | --- | --- | --- | 1 |
| Slight pelvic dilatation of kidney (unilateral) | 1 | 1 | 3 | --- |
| (bilateral) | 1 | --- | --- | --- |
| Ureter dilated (bilateral) | --- | --- | 1 | --- |

a Data were obtained from Tables 7 and 8 on pages 30-31 and from individual data on pages 104-105 and 112 of the study report.
b All findings denoted by this subscript were observed in the same fetus (Fetus B, Doe #45)..
c All findings denoted by this subscript were observed in the same fetus (Fetus A, Doe #57).
--- No animals affected (i.e., zero incidence)

3. **Skeletal examination:** There were no treatment-related skeletal malformations or variations (Table 9a). Extreme reduction in ossification of the skull bones was observed in one 7.5 mg/kg/day fetus and one 15 mg/kg/day fetus.

An increased incidence of 13 bilateral lumbar ribs (1 normal length and 1 short) was observed at 7.5 and 15 mg/kg/day (4.2-11.5%) compared to concurrent (2.1%) controls. However, the increase was not statistically significant, and a treatment-related effect on any of the other subtypes of extra lumbar rib was not observed. Additionally, a minor increase in the incidence of 13$^{th}$ unilateral thoracic rib (arising from the 10$^{th}$ thoracic vertebra) was observed at 15 mg/kg/day (1.6%) compared to concurrent controls (0%). However, this increase was also not statistically significant. The incidences of both of these variations fell within the range of historical controls for extra 13$^{th}$ rib (0.0-27.8% bilateral; 4.0-37.9% unilateral).

Several other variations were observed at an increased incidence over controls, including: (i) partially ossified frontals at 7.5 and 15 mg/kg/day (6.4-13.1% fetuses) compared to controls

(2.1%); (ii) partially ossified interparietals at 15 mg/kg/day (6.5% fetuses) compared to controls (1.1%); (iii) not ossified interparietals at 15 mg/kg/day (3.3% fetuses) compared to controls (0%); (iv) 7th lumbar vertebra not ossified at 15 mg/kg/day (1.6%) compared to controls (0%); (v) extra center of ossification between the 5th and 6th sternebrae at 7.5 and 15 mg/kg/day (1.1-1.6%) compared to controls (0%); and (vi) pelvic girdle articulation moved anteriorally in all treated groups (1.0-3.3%) compared to controls (0%). However, these findings were minor in incidence and were not significantly different from the controls. All other skeletal malformations and variations were unrelated to dose.

Mean scores for ossification of the bones of the hand (*manus*) and foot (*pes*) are included in Table 9b. Mean scores for ossification of the *manus* in all treated groups (1.77-1.95) were comparable to controls (1.88), when the single 7.5 and 15 mg/kg/day fetuses with extreme reduction in ossification were excluded. Similarly, mean scores for ossification of the *pes* in all treated groups (1.75-1.95) were comparable to controls (1.88).

**TABLE 9a. Selected skeletal findings (# [%] fetuses affected)** [a]

| Observation | Dose (mg/kg/day) | | | |
|---|---|---|---|---|
| | 0 | 2.5 | 7.5 | 15 |
| **No. Fetuses (litters) examined** | 94 (15) | 102 (15) | 94 (15) | 61 (9) |
| Malformations (major defects) | | | | |
| **Malformed skull bones – extreme reduction in ossification** | --- | --- | 1 [1.1] [b] | 1 [1.6] [c] |
| Variations (minor defects and variants) | | | | |
| **Ribs, extra** | | | | |
| **Lumbar, 13 unilateral, short length** | 4 [4.2] | 9 [8.8] | 4 [4.2] | 6 [9.8] |
| **Lumbar, 13 unilateral, normal length** | 3 [3.2] | 4 [3.9] | 1 [1.1] | 1 [1.6] |
| **Lumbar, 13 bilateral, short length** | 1 [1.1] | 4 [3.9] | 4 [4.2] | 3 [4.9] |
| **Lumbar, 13 bilateral, normal length** | 3 [3.2] | 10 [9.8] | 14 [14.9] | 9 [14.7] |
| **Lumbar, 13 bilateral, 1 normal/1 short length** | 2 [2.1] | 2 [2.0] | 4 [4.2] | 7 [11.5] |
| **Thoracic, 13 unilateral, normal length, arises from 10th thoracic vertebra** | --- | --- | --- | 1 [1.6] |
| **Frontals, partially ossified** | 2 [2.1] | 2 [2.0] | 6 [6.4] | 8 [13.1] |
| **Parietals, partially ossified** | 6 [6.4] | 4 [3.9] | 3 [3.2] | 4 [6.5] |
| **Occipitals, partially ossified** | 2 [2.1] | --- | --- | 2 [3.3] |
| **Hyoid, partially ossified** | 14 [14.9] | --- | 2 [2.1] | --- |
| **Interparietal, partially ossified** | 1 [1.1] | 2 [2.0] | --- | 4 [6.5] |
| **not ossified** | --- | --- | --- | 2 [3.3] |
| **Lumbar vertebra (7th), not ossified** | --- | --- | --- | 1 [1.6] |
| **Sternebra(e) – extra center of ossification between 5th and 6th** | --- | --- | 1 [1.1] | 1 [1.6] |
| **Pelvic girdle – articulation moved anteriorally** | --- | 1 [1.0] | 3 [3.2] | 2 [3.3] |

a Data were obtained from Tables 7 and 8 on pages 30 and 32-36 and from individual data on pages 104 and 112 of the study report.

b All findings denoted by this subscript were observed in the same fetus (Fetus B, Doe #45)..

c All findings denoted by this subscript were observed in the same fetus (Fetus A, Doe #57).

--- No animals affected (i.e., zero incidence)

**TABLE 9b. Scores for skeletal ossification of the hand (*manus*) and food (*pes*) [a]**

| Interval | Dose (mg/kg/day) | | | |
|---|---|---|---|---|
| | 0 | 2.5 | 7.5 | 15 |
| No. Fetuses (litters) examined | 94 (15) | 102 (15) | 94 (15) | 61 (9) |
| *Manus* score (# [%]) | | | | |
| 1 | 17 [18.1] | 14 [13.7] | 23 [24.5] | 6 [9.8] |
| 2 | 74 [78.7] | 86 [84.3] | 68 [72.3] | 51 [83.6] |
| 3 | --- | 2 [2.0] | 2 [2.1] | 3 [4.9] |
| 4 | 3 [3.2] | --- | --- | --- |
| Outside range | --- | --- | 1 [1.1] [b] | 1 [1.6] [c] |
| Mean *manus* score [d] | **1.88** | **1.88** | **1.77** | **1.95** |
| *Pes* score (# [%]) | | | | |
| 1 | 13 [13.8] | 10 [9.8] | 23 [24.5] | 3 [4.9] |
| 2 | 79 [84.0] | 91 [89.2] | 70 [74.5] | 57 [93.4] |
| 3 | 2 [2.1] | 1 [1.0] | --- | --- |
| Outside range | --- | --- | 1 [1.1] [b] | 1 [1.6] [c] |
| Mean *pes* score [d] | **1.88** | **1.91** | **1.75** | **1.95** |

a Data were obtained from Table 8 on page 36 of the study report. Scale for semi-quantitative assessment of skeletal ossification of the *manus* and *pes* was obtained from Appendix 6 on page 43 of the study report (1 = good; 5 = poor), included as an Attachment to this DER.
b Single fetus (Fetus B, Doe #45) with multiple malformations and extreme reduction in ossification.
c Single fetus (Fetus A, Doe #57) with multiple malformations and extreme reduction in ossification.
--- No animals affected (i.e., zero incidence)
d Calculated by the reviewers from data presented in this table, excluding the two fetuses outside of the range.

## III. DISCUSSION AND CONCLUSIONS

**A. INVESTIGATORS' CONCLUSIONS:** It was concluded that the maternal and developmental LOAELs were 15 mg/kg/day based on decreased maternal food consumption and body weight loss during the dosing period. Two does at this dose level had fur balls in the stomach, and one of these animals had stomach lesions; these findings were considered to be due to non-specific stress in rabbits in poor condition. Fetuses in this group showed evidence of embryotoxicity as reflected by high post-implantation loss and an increase in the number of fetuses with retarded ossification of the skull bones. The increased incidence of extra lumbar ribs in all treated groups fell within the range of historical controls. The multiple defects noted in a single fetus at 7.5 mg/kg/day and another at 15 mg/kg/day were considered unrelated to treatment because a similar suite of malformations were noted in historical control data and because the malformations at 7.5 mg/kg/day were more severe than those at 15 mg/kg/day.

**B. REVIEWER COMMENTS**

1. **Maternal toxicity:** At 7.5 mg/kg/day, one doe (#52) aborted part of its litter on GD 20. At 15 mg/kg/day, one doe (#70) was killed *in extremis* after observations that the animal had not been eating or drinking and that it had lost weight and was in poor condition. No other maternal deaths could be attributed to treatment. Loose feces on the cage floor and/or fur of the animals was observed in 2/15 rabbits at 7.5 mg/kg/day and 4/15 rabbits at 15 mg/kg/day.

These findings were observed only once per female, except for one doe at 7.5 mg/kg/day for which the observation was made on 2 days and one doe at 15 mg/kg/day for which loose feces was noted on three days. The finding of loose feces was considered treatment-related but non-adverse.

At 15 mg/kg/day, maternal body weight gains were decreased during the treatment interval (-79 g treated vs 48 g controls) and for the overall (GD 0-29) study, both when uncorrected for (149 g treated vs 230 g controls) and when corrected for (-158 g treated vs -55 g controls) gravid uterine weights. Additionally at this dose, maternal food consumption was decreased by 22% (not significant) during treatment. In two of the females examined at 15 mg/kg/day, the stomach was found to contain a fur ball and was otherwise empty or contained little food. Only a single female at 7.5 mg/kg/day had little to no food in the stomach. Additionally at 15 mg/kg/day, one of the aforementioned does had dark pitted areas on the mucosal surface of the glandular portion of the stomach.

**The maternal LOAEL is 15 mg/kg/day based on decreased corrected and uncorrected body weight gains and food consumption. The maternal NOAEL is 7.5 mg/kg/day.**

2. **Developmental toxicity**

a. **Deaths/resorptions:** The number of early intrauterine deaths was higher at 15 mg/kg/day than controls (36 deaths; 31.0%) compared to controls (11 deaths; 10.4%). Similarly, the number of late intrauterine deaths was increased at this dose (19 deaths; 16.4%) compared to controls (1 death; 1.0%). Complete litter resorptions were significantly higher ($p \leq 0.05$) at 15 mg/kg/day, occurring in 5/14 does compared to 0/15 controls. These findings resulted in a significantly increased ($p \leq 0.01$) post-implantation loss at 15 mg/kg/day (45.5% vs 13.1% controls); a decreased number of litters (9 vs 15); and a decreased total (61 vs 94) and mean (4.0 vs 6.5; $p \leq 0.05$) number of live fetuses.

b. **Altered growth:** There were no treatment-related effects on growth or development of the fetuses. Fetal body weights and litter weights of the treated groups were comparable to controls. Reduced/delayed ossification was observed in several bones in the skeleton (skull, vertebrae, and sternebrae) at an increased incidence over controls. However, these findings were minor in incidence and were not significantly different from the controls. Furthermore, mean scores for ossification of the *manus* and *pes* in all treated groups were comparable controls.

c. **Developmental variations:** There were no treatment-related external, visceral, or skeletal variations. All findings were unrelated to dose, minor in incidence, and/or not significantly different from the controls.

d. **Malformations:** There were no treatment-related external, visceral, or skeletal malformations. Two fetuses, one at 7.5 mg/kg/day and another at 15 mg/kg/day, had multiple abnormalities; however, historical control data showed that similar findings were previously noted in individual fetuses (e.g., cleft palate, gastroschisis, malformed eyes, and

shortened/flexed limbs with reduced number of digits). The following findings were observed in a single fetus at 7.5 mg/kg/day (Fetus B, Doe #45): (i) upper jaws shortened; (ii) cleft palate; (iii) right eye open; (iv) left eye microphthalmia; (v) polex absent (bilateral); (vi) clubbed right hindfoot; (vii) abnormally shaped left hindlimb with a reduced number of digits; (viii) encephalocoele; (ix) gastroschisis; and (x) extreme reduction in ossification of the skull bones. The following findings were observed in a single fetus (Fetus A, Doe #57) at 15 mg/kg/day: (i) shortened upper jaw; (ii) bilateral clubbed hindfeet; (iii) shortened left forearm; (iv) flexion of forefoot; (v) encephalocoele; (vi) gastroschisis; and (vii) extreme reduction in ossification of the skull bones.

**The developmental LOAEL is 15 mg/kg/day based on decreased number of live fetuses, complete litter resorptions, and increased post-implantation loss. The developmental NOAEL is 7.5 mg/kg/day.**

This study is classified **acceptable/guideline** and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in rabbits.

C. **STUDY DEFICIENCIES:** The following deficiency was noted but does not affect the acceptability or the conclusions of this DER:

- Does were treated only during GD 6-19 instead of GD 6-28. However, this dosing duration covered the period of organogenesis, and was considered acceptable according to the guidelines established shortly after the completion of this study (Pesticide Assessment Guideline §82-2, Subdivision F; November, 1984).

# ATTACHMENT

The following is page 43, Appendix 6 of the study report.

## APPENDIX 6

### SCALE FOR ASSESSMENT OF OSSIFICATION OF DIGITS

Scale

| | |
|---|---|
| 1 (good) | Metacarpals/metatarsals and 1st, 2nd and 3rd rows of phalanges fully ossified. |
| 2 | Metacarpals/metatarsals and 1st and 3rd rows of phalanges fully ossified, some of 2nd row not ossified. |
| 3 | Metacarpals/metatarsals fully ossified. All 1st and 3rd row present, the majority being fully ossified, most of 2nd row not ossified although occasional phalanx may be partially ossified. |
| 4 | One metacarpal or metatarsal may be partially ossified, remainder of the metatarsals or metacarpals fully ossified. 2nd row of phalanges not ossified, most of 1st and 3rd rows of phalanges fully ossified but a few partially ossified. |
| 5 | One metacarpal or metatarsal partially ossified or not ossified, remainder of metatarsals and metacarpals fully ossified. 2nd row of phalanges not ossified, occasional phalanges in 1st and 3rd row not ossified, remainder partially ossified. |